

*(From the Department of Clinical Biochemistry, Karolinska Sjukhuset, Stockholm, Sweden).*

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# SOME EXPERIMENTS ON PARTLY HYDROLYSED BACTERIAL DEXTRAN

By  
BERTIL ÅBERG

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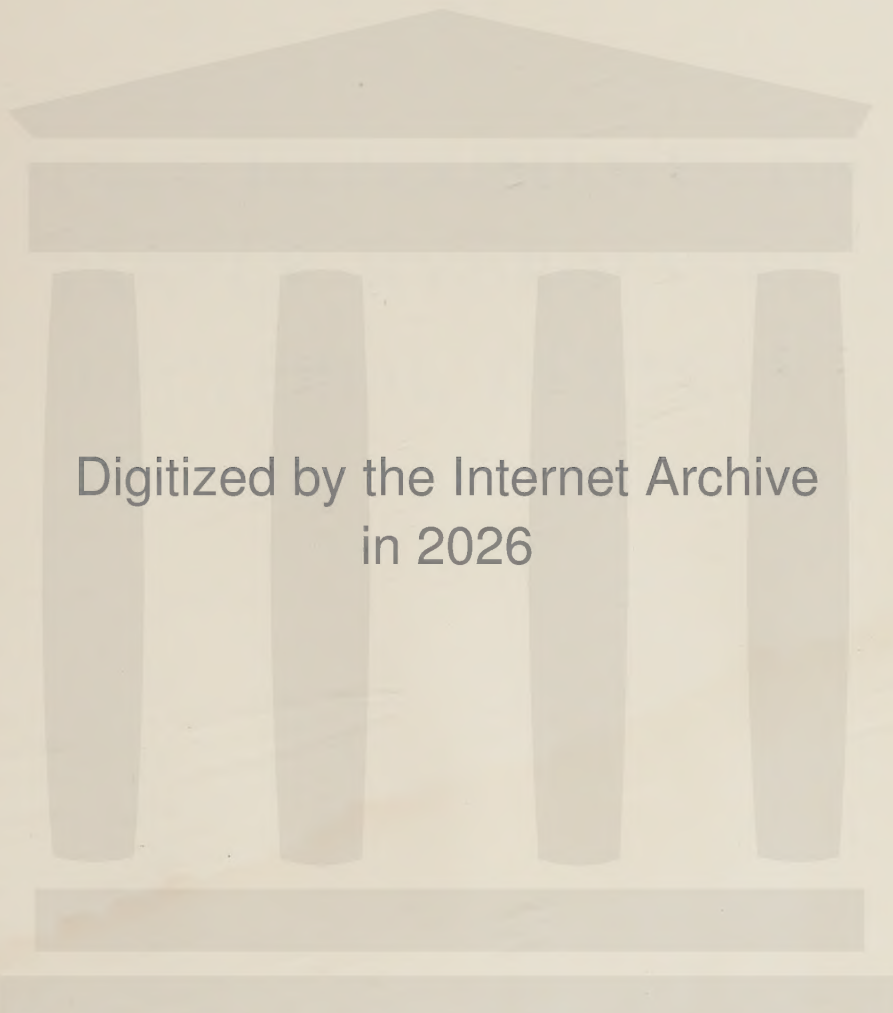
SOME EXPERIMENTS ON PARTLY HYDROLYSED  
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## PREFACE.

The author wishes to thank his colleagues for their very excellent collaboration which made this work on dextran possible.

I wish to express to Bertil Swedin, M. D., my deepest gratitude for facilities which he provided at the Department of Clinical Biochemistry, Karolinska Sjukhuset, and for the encouraging interest which he has taken in the investigations.

Professor John Hellström, head of the Surgical Clinic at Karolinska Sjukhuset as well as Professor Gösta Häggqvist, head of the Histological Department, Karolinska Institutet, have taken a great interest throughout this investigation and it is with great pleasure that I thank them for their kindness.

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I also wish to thank Dr. P. Cannon, M.Sc., M.B. and Dr. I. Forsythe, M.D., M.R.C.P. for revising the English translations.

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INTRODUCTION.

Since many years dextran has been widely used as a plasma volume expander, but its fate in the body after intravenous administration has not been completely known. The aim of the experiments reviewed here has been to study the elimination of dextran as well as its effect on the plasma proteins and on the erythrocyte sedimentation rate.

The author bases this dissertation on the following publications in which he has taken part:

- I. Friberg, U., Graf, W. & Åberg, B.: On the Histochemistry of Partly Hydrolysed, Bacterial Dextran. *Scandinav. J. Clin. & Lab. Investigation* 3, 221, 1951.
- II. Swedin, B. & Åberg, B.: On Dextran in Spleen, Liver, and Muscle after Intravenous Injection into Rabbits. *Ibid.* 4, 68, 1952.
- III. Troell, L. & Åberg, B.: The Carbohydrate Content of the Gastric Juice after Intravenous Administration of Partly Hydrolysed, Bacterial Dextran to Hospital Cases. *Ibid.* 4, no. 4, 1952.
- IV. Ryttinger, L., Swedin, B. & Åberg, B.: The Effect of Dextran on the Erythrocyte Sedimentation Rate in Man. *Ibid.* 4, no. 4, 1952.
- V. Åberg, B.: Break Down of Dextran by Human Faeces. *Ibid.* 4, no. 4, 1952.

References are made with the Roman figures given above.

## I. ESTIMATION OF DEXTRAN.

When studying the ultimate fate of intravenously infused dextran, the first problem to arise and the one of paramount importance is the qualitative and quantitative determination of dextran. Since the introduction of partly hydrolysed dextran as a "plasma substitute" by *Grönwall & Ingelman* (1944), a number of methods have been employed to estimate the concentration of dextran in various body fluids or organs. It is the aim of this chapter to review these methods briefly.

In pure aqueous solutions, dextran can be determined e.g. by estimating the reducing power before and after complete acid hydrolysis. *Klevås* (1944) elaborated such a technique in order to estimate dextran in blood and plasma. The method is, however, laborious and too time consuming to be used as routine method.

Polarimetry has been used much. The mean value of the specific rotation of partly hydrolysed dextran was found to be  $+185^\circ$  (*Grönwall & Ingelman* 1945). Improved methods of determining the dry weights of the substance gave higher values, e.g. dextran isolated from urine  $+196^\circ$  (*Ingelman & Halling* 1949) or for fractions of Swedish dextran  $+204^\circ$ — $+229^\circ$  (*Weissberg & Isbell* 1951). Polarimetry can be used for determinations on e.g. urine or gastric juice.

*Hint & Thorsén* (1947) elaborated a technique in which dextran is precipitated in alkaline solution by copper, and the remaining copper estimated colorimetrically as cupramate after treatment with sodium diethyldithiocarbamate. The dextran concentration of the sample is calculated from a standard curve. By varying the amount of copper added, high concentrations of dextran may be determined. Dextran concentrations less than 200 mg. per 100 ml. should however not be determined with this method. The blank value being fairly high, it is necessary to correlate the dextran value obtained to the amount of dry substance when the method is applied to homogenates of organs. It is also necessary to make comparisons, using homogenates of organs from animals which have not received any dextran.

The accuracy of the *Hint & Thorsén* method calculated from a number of double determinations was found to be  $\pm 3.0$  per cent at a concentration of 1.00 g. per 100 ml. (I).

*Gurin & Hood's* modification (1939) of *Dische's* carbazol reaction has been used much. Employing standard conditions, viz. a constant time of heating and boiling a series of standard solutions simultaneously, the accuracy of the method was found to be  $\pm 3.6$  per cent at a dextran concentra-

tion of 100 µg. per 100 ml. (III). Care was taken to dilute the tests so as to contain less than 200 µg. of carbohydrates per ml.

In recent times anthrone has been used as a reagent for dextran (*Durham et al. 1950; Bloom & Willcox 1951*). Like the carbazol in the Dische method, anthrone gives a colour when heated in sulphuric acid with hexoses or pentoses. The reaction is stated to be due to the formation of hydroxymethylfurfural and furfural respectively (e.g. *Zipf & Waldo 1952*). In the anthrone methods described, however, the heat necessary for the reaction is liberated by pouring sulphuric acid containing the reagent into the test solution. In order to get constancy, it is necessary therefore to have test tubes of constant volume and thickness (*Zipf & Waldo 1952*).

In urine, *Goldenberg et al. (1947)* precipitated dextran with copper and redissolved the precipitate in distilled water. The carbohydrate content was estimated with the carbazol method.

Serological methods have been used by other authors in order to trace dextran in various organs (e.g. *Bull et al. 1949*). We have not been able to use these techniques as the bacteria used in manufacturing dex-

tran (*Leuconostoc mesenteroides*) has not been at our disposal.

The periodic acid—leucofuchsin technique described by *McManus (1946)* and *Hotchkiss (1948)* was applied in histochemical work by *Friberg, Graf & Aberg (1, 1952)*. The method gives good results (*Mowry et al. 1952*) provided that the alcohol content of the reagents is high enough. However, it is necessary to stress, that all substances with 1,2-glycol groups or with the equivalent structure in which one of the hydroxyl groups is replaced by an amino group will give a colour reaction.

None of these methods mentioned above is specific for dextran except perhaps the serological ones. However, care was taken in this study to compare the results obtained in the dextran experiments with such from animals which had not been treated with dextran.

In order to identify the substance shown to be present in gastric juice after intravenous infusion of dextran the substance was isolated (III). Specific rotation, rate of hydrolysis on boiling with acid and chromatography after acid hydrolysis showed that the substance probably was dextran.



## II. THE FATE OF DEXTRAN IN THE BODY.

### A. EXPERIMENTS ON ANIMALS.

#### 1. *In vivo* experiments.

Different strains of *Leuconostoc* bacteria produce dextran with different properties when grown on saccharose. The ratio of 1,6- to 1,4-glucosidic linkages as well as the mean molecular weight distribution are the most important variables. It is not clear how these differences influence the behaviour of different dextran types in the mammalian body. The various manufacturers use different strains of *Leuconostoc* and stop the partial acid hydrolysis of the raw dextran at different stages. It is furthermore stated that the manufacturer in Sweden now and then changes the procedure of preparation of dextran to be used clinically (Grönwall 1949). It is therefore not always possible to compare the results obtained in experiments with dextrans of different manufacture.

When the present Swedish dextran hydrolysate for clinical use, "Macrodex", was intravenously injected into rabbits, between 20 and 30 per cent of the dose administered was excreted in the urine. The bulk of urinary dextran appeared within 12 hours after the injection (polarimetric estimation) and only very small amounts of dextran appeared in the urine after 24 hours had elapsed (Åberg 1952, unpublished). The same results were earlier obtained by Thor-

sén (1949) on "Dextran Ph", by Bull *et al.* (1949) on British dextran and by Turner *et al.* (1949) on "Macrose" prepared in the U.S.A. Ingelman & Halling (1949) isolated the dextran excreted in the urine and found that its molecular weight did not exceed 36,000.

In the blood, dextran was found to be present for 4 to 5 days after a single dose. The blood plasma concentration was found to decrease at a constant rate after the first 6 hours (Grönwall & Ingelman 1945, Thorsén 1949, Bull *et al.* 1949). Thorsén (1949) found that the rate of decrease was mainly dependent upon the weight of the animal. According to some authors, the dextran which is not excreted in the urine, will be slowly broken down in the body (Ingelman 1949, Hildebrandt 1950). Ingelman (1949) found that dextran disappeared more rapidly from the blood in animals with experimental thyrotoxicosis than in normal ones. Furthermore, according to Ingelman (1947) and Thorsén (1949) it seemed probable that dextran was broken down in the body, as no dextran could be detected in organs of animals who had been given dextran intravenously in large quantities.

It has been shown by Thorsén (1948, 1950) that dextran passes from the blood stream via the capillary walls into blisters produced by burning. Grotte *et al.* (1951)

showed, that dextran appeared in the thoracic lymph of a dog when it had been given dextran intravenously. In their experiments, the dextran concentration of the thoracic lymph decreased in parallel with that of the blood plasma.

*Bull et al.* (1949) used a dextran hydrolysate manufactured in England. Of the dose administered, about 25 per cent was eliminated through the kidneys. No histological changes were found in the liver, kidney, spleen, heart muscle, thyroid gland or adrenals of rabbits injected with such dextran. By use of antisera against *L. mesenteroides* prepared in rabbits, *Bull et al.* (1949) found that some of the dextran injected accumulated for a long time in the reticuloendothelial system of the rabbit.

*Goldenberg et al.* (1947) found marked but transient histological changes in the renal glomeruli and tubules from rabbits, which had been intravenously injected with an American dextran hydrolysate. The mean molecular weight of their dextran was probably higher than that of the Swedish dextran, as only 6 per cent of the dose administered was excreted in the urine. Histological changes were found by *Turner et al.* (1949) in the liver and kidney from dogs intravenously infused with dextran ("Macro-rose"). *Grönwall & Ingelman* (1945) found that high molecular dextran, "raw dextran", when intravenously infused produced lethal changes in the kidneys of rabbits.

That dextran is metabolized by mice has been shown by *Cargill & Brunner* (1951), who administered a dextran labelled with radioactive carbon ( $C^{14}$ ) and found that the mice expired  $C^{14}O_2$ . A number of experiments have been performed on rats and mice. These animals, however, show an allergic reaction towards dextran with

cyanosis and oedema of the nose and the legs (*Voorhees et al.* 1951, *Morrison et al.* 1951). It is the author's opinion that the results obtained from animals showing such allergic reactions, cannot be used in a discussion concerning the fate of dextran in the human body.

*Gray et al.* (1951) have presented indirect evidence that "Macrodex" is broken down to glucose in phlorizinized dogs. These authors studied the dextrose/nitrogen ratio in the urine before and after infusion of "Macrodex", and found an increase in this ratio.

In a perfusion experiment with camula inserted into the blood vessels, *Engstrand & Åberg* (1950) were not able to detect any uptake of dextran in rabbit's liver. According to their results, the uptake, if any, amounts to about 0.5 mg. of dextran per ml. of blood passing through the liver. They stated, however, that there was an excretion of dextran into the stomach as well as into the intestine of rabbits who had been given dextran intravenously. The same results were obtained with a cat. In the cat, no dextran was found in the bile.

*Gray et al.* (1951) studied the gastrointestinal excretion of "Macrodex" in dogs. During 24 hours after the intravenous infusion, less than 0.1 per cent of the intravenously administered "Macrodex" was found in the gastro-intestinal tract.

## 2. *In vitro* experiments.

Animal experiments hitherto published have not revealed with sufficient completeness the ultimate fate of intravenously administered dextran. This led several investigators to make *in vitro* experiments. It was expected that amylase might be able to break down dextran. *Grönwall & Ingel-*



man (1945) stated that dextran is very slightly split by starch splitting enzyme. Ingelman (1947) found that amylases did not break down dextran. Bull *et al.* (1949) incubated dextran with blood and found a very slight decrease in the dextran content. Engstrand & Åberg (1950) incubated dextran with human blood as well as with aqueous extracts of liver tissue and glycerol extracts of pancreas and pig's intestine. No signs of any break down of the dextran were found. The same result was obtained by Bull *et al.* (1949) on incubating dextran with extracts of guinea pig pancreas and liver or with suspensions of finely ground rabbit lymph gland, spleen and liver.

Schmitz (1951a) stated that liver-, spleen- and kidney-homogenates from the rat were able to "dehydrate" "Macrodex", as judged by a reaction with triphenyltetrazoliumchloride. Viscosimetry, however, revealed no break down of the "Macrodex", when it was incubated with various organs of the rat. A decrease in the viscosity occurred according to Schmitz (1951 b) when dextran was incubated for 100 hours with white blood cells of a horse. The content of glucose in the incubate did not increase.

Grönwall & Persson (*cit.* Grönwall 1951) found that dextran was altered in the tissues so that it could be stained with leuco-fuchsin without previous treatment with periodic acid. These authors presumed that this was due to oxidation of dextran in the tissues.

In conclusion, the ultimate fate of dextran in the body is not known with any certainty except for that part of the dextran molecule which is capable of passing through the renal barrier into the urine. Whether the remaining part of the injected

dextran is metabolized in the tissues is not known, as far as can be judged from the literature cited above.\* However, it is known that dextran, tagged with radioactive carbon and injected into mice is to some degree broken down to carbon dioxide. The mechanism of this break-down has not been studied as far as the author knows.

In 1950, Friberg, Graf & Åberg (I) started to investigate the fate of Swedish dextran, injected intravenously into rabbits. The animals were injected with a standard dose of dextran (1 g. per kg. of body weight) and killed 18 hours later. By that time, the bulk of the dextran excreted in the urine should have left the body, and so the remaining dextran might possibly be found in the tissues. The periodic acid — leucofuchsin technique elaborated by Hotchkiss (1948), which had earlier been applied to heparinmonosulphuric acid (Jorpes, Werner & Åberg 1948, Jorpes, Gardell, Werner & Åberg 1948, Friberg, Graf & Åberg 1951), was found to be applicable to dextran *in vitro* (I).

*Effect of dextran on connective tissue and white blood corpuscles:* From the behaviour of a 10 per cent dextran solution injected as a wheal into a guinea pig it was shown in the present investigation (I) that a complete absorption from the wheal would take at least 8 days. A pronounced accumulation of white blood corpuscles occurred around the wheal. This reaction

\*) Gray *et al.* (1951) have presented evidence that dextran is metabolized to glucose in the phlorizinized dog. They injected Macrodex intravenously and found an increase of reducing substance in the urine. The evidence presented above, viz. that dextran is broken down and/or "dehydrated" or oxidized in the tissues was not however connected with an increase of reducing substance (glucose) *in vitro*.

appeared within 20 minutes after the injection and reached its maximum at 12 hours and then gradually disappeared. When dextran of a mean molecular weight of 28,000 was used, the chemotactic effect was about the same as that produced by a dextran with a mean molecular weight of 130,000. The leucocytes seemed to show a stronger colouring than normal leucocytes, a fact that might be interpreted as due to the probable storage of dextran within the cells. Summing up, dextran is slowly absorbed from the connective tissue, and like e. g. glycogen (*Chambers & Grand 1936, Sheldon cit. Mc Cutcheon 1946*) it exerts a positive chemotaxis in leucocytes who probably store dextran.

*Effect of dextran on spleen:* As cited above, *Bull et al. (1949)* had been able to show a storage of dextran in the reticulo-endothelial tissue. In the present investigation it was found (I), that in spleens from animals injected with dextran, there occurred an increased number of cells, stainable with the *Hotchkiss* technique. It was concluded, that this could be interpreted as a sign of dextran carrying leucocytes being stored in the spleen. The storage of dextran in the spleen was further studied (II). In these experiments "Macrodex" was intravenously injected into rabbits and the animals were killed 18 hours after the injection. When the spleens were examined chemically, they showed a definite accumulation of dextran. A chronic toxicity study of dextran, performed by *Friberg, Graf & Åberg (1952)* showed that when 1 g. of "Macrodex" per kg. body weight was administered intravenously into rabbits every day for 3 1/2 months and the animals killed, the spleens were 3—4 times larger than those from normal animals.

*Effect of dextran on the kidneys:* It has already been mentioned, that *Grönwall & Ingelman (1945)* injected high molecular, "raw dextran", intravenously into rabbits and found that it killed the animals. When sectioned, the kidneys showed marked necrotic changes. Furthermore the low molecular part of intravenously administered dextran leaves the body through the kidneys. In the present investigation (I) it was thus decided to study the kidneys histochemically after dextran infusions. The ordinary Swedish clinical dextran is stated to have a mean molecular weight of about 75,000 with a range between 20,000 and 200,000 (*Ingelman 1951*). In the present investigation (I) fractions with the following mean molecular weights were used: 28,000; 40,000; 75,000; 130,000 and 400,000. The dose was 1.0 g. of dextran per kg. of body weight and the substance was administered intravenously to rabbits as 10 per cent solution in distilled water. Animals were killed at different times after the injection, the kidneys homogenized and the dextran content of the homogenate estimated according to *Hint & Thorsén (1947)*. The percentage of dry substance in the homogenates was also determined. It was found, that a transient accumulation occurred with all the dextran fractions used. This accumulation was most pronounced for the fractions with a low molecular weight which allowed them to pass into the urine. However, even those dextran fractions which could not be traced in the urine with the method used (polarimetry) showed a small but significant transient accumulation in the kidney. The histological picture showed, not only the presence of dextran in the glomerular capillary loops as well as in the collecting tubules, but abundant amounts

of dextran granules in the epithelial cells of the proximal convoluted tubules as well as in the loops of Henle. However, it was not possible to draw any conclusions about the significance of these granules, viz. if they signify a reabsorption, an excretion or storage.

*Effect of dextran on the liver, the lung, the muscles:* In the present investigation, there was histochemically no sign of any storage of dextran in the liver, the lungs or the heart muscle in rabbits which had been injected with dextran (I). Furthermore no storage of dextran was found in the liver or the femoral muscle of rabbits injected with dextran ("Macrodex") and killed 18 hours after the administration (II).

#### *Discussion.*

From the animal experiments recorded above, one might draw the following conclusions regarding the fate of dextran in the organism. The low molecular portion of the intravenously administered dextran preparation will be excreted in the urine. The quantitative estimation of urinary dextran seems uncertain, as no good methods exist to estimate the dextran concentration in the urine. However, most investigators agree, that about 25 per cent of "Macrodex" is excreted in the urine. Changes in the manufacturing process might change this figure in the future. When passing through the kidneys, the dextran is transiently accumulated in the tubular cells. Whether this accumulation affects the function of the tubules is not known with any certainty as far as the author knows. The effect on the kidneys of "Macrodex" cannot be of any grave nature, as animals injected with large doses of this dextran preparation are able to live a long time afterwards with apparently good

health. It is noteworthy, that even large molecules of dextran, which cannot pass out into the urine, are transiently stored in the tubular cells (I). The mechanism of this is not known.

Some of the dextran is stored in the reticuloendothelial system for a long time. The present investigations have confirmed that dextran accumulates in the spleen (I, II). It has also been shown (I) that dextran exerts a chemotactic influence on leucocytes and that these cells are probably able to take up some dextran. The chemotactic influence of low molecular dextran was about the same as that of the high molecular fraction.

The amounts stored in the spleen and probably in the leucocytes\* (I, II) are not, however, of sufficient magnitude to explain the ultimate fate of all the injected dextran. The results of *Engstrand & Åberg* (1950) that dextran is excreted into the gastro-intestinal tract seem more promising. The methods used in that investigation were however as will be shown, not good enough to make it possible to draw any conclusions as to the quantitative importance. It was found therefore, necessary to change the methods of analysis and to try to study the gastro-intestinal excretion in man.

#### B. EXPERIMENTS ON MAN.

##### 1. *In vivo experiments.*

Numerous reports have appeared which deal with the effect of dextran as a plasma volume expander. These reports will not be

\*) This uptake does not seem to occur with human white blood cells *in vitro*, as no change in the dextran concentration was observed when dextran ("Macrodex") was incubated with human blood (*Engstrand & Åberg* 1950). Nor could the dextran have been broken down to any large extent during the 96 hours incubation, as the substance could still be quantitatively precipitated with copper in alkaline solution after that time.



reviewed here except where they also deal with the problem of the fate of dextran in the human body.

As in animals, "Macrodex" disappears slowly from the blood when intravenously injected into man (*Ingelman 1947*). Between 23 and 31 per cent of injected "Macrodex" of present manufacture is excreted in the urine (III). The rest of the dextran in the blood will decrease to 0 during the 4—5 days following the infusion (*Ingelman 1947*).

The ultimate fate of the dextran not excreted in the urine is not known. The findings recorded above have been applied to man, and it has generally been presumed, that dextran behaves in man as it does in animals.

In 1949, *Engstrand*, as a result of his earlier work on the permeability of liver sinusoids, suggested that the route of the elimination of dextran should be investigated.

In a case of paralytic ileus, *Engstrand & Åberg* (1950) found that the gastric juice received through a gastric fistula contained large amounts of dextran. When the animal experiments, started by these authors, were continued, it was found that the gastric juice received from rabbits was unreliable. With carbohydrate estimation according to *Gurin & Hood* (1939), the presence of large amounts of carbohydrate was found in the gastric juice of animals who were fasting since several days. Inspection of the gastric content showed, that there was in all cases lots of food in the stomach. When the stomach was cleaned under anaesthesia and dextran given, insignificant amounts of carbohydrate appeared in the gastric juice during the 3—6 hours following the infusion (*Åberg 1951, unpublished*).

As dextran is used as a therapeutic mea-

sure in man against shock it was thought that it would be better to study the gastric secretion in man.

Such a study was performed by *Troell & Åberg* (III). All the patients were males and investigated prae- or postoperatively. They received "Macrodex" intravenously and had fasted 12 hours before the gastric juice was recovered through suction. The "gastric juice" in the experiment actually consisted of saliva, oesophageal mucus and the gastric secretion. Free hydrochloric acid was always present and in only one case was haemoglobin present. Saliva obtained from men intravenously infused with "Macrodex" did not contain any dextran (*Åberg 1951, unpublished*). Some patients, praoperatively investigated, suffered from duodenal ulcer, and the possibility naturally exists that serous fluid from the ulcer had been mixed with gastric juice. The gastric contents were recovered during 5—6 days after the infusion when possible. When food was given, it contained as little carbohydrate as possible and the patient fasted for at least 12 hours before the tube was placed in the stomach.

The carbohydrate content of the gastric juice was estimated on a trichloroacetic filtrate according to *Dische* as modified by *Gurin & Hood* (1939). An outflow of carbohydrate occurred in the gastric juice in most cases. The magnitude of the carbohydrate output was greatest 12 to 48 hours after the infusion of dextran. As a control the mean value of carbohydrate in the gastric juice was estimated in 28 healthy subjects.

In order to try to prove if the carbohydrate was dextran, it was isolated from 3 portions of juice from 2 patients. Chemical analyses showed that the isolated substance was probably dextran (III).

Burson & Bloom (1951) could only find insignificant amounts of dextran in the gastro-intestinal tract after intravenous administration of dextran to man. However, these authors only studied the gastro-intestinal content of dextran during some hours after the administration.

It remains to be shown if the route of elimination of dextran in healthy man is via the gastro-intestinal tract. It is not possible to make any conclusions regarding the quantitative elimination of dextran this way. This depends largely on the impossibility to suck the stomach empty — haemorrhages then occur. Furthermore, great variations exist in the amount of gastric juice secreted in the same patient from time to time as well as when patients are compared with each other.

## 2. *In vitro* experiments

Engstrand & Åberg (1950) were not able to detect any dextran in the faeces from patients who had been intravenously infused with dextran. When dextran was incubated with faeces *in vitro* it was found to disappear rapidly. It was supposed, that this break down of dextran was due to bacterial action. Hebre & Sery (1952) have confirmed that faecal bacteria break down dextran. They were able to isolate a number of anaerobic bacteria which possessed this ability.

Ingelman (1948) found that extracts from *Cellvibrio fulvia* were able to break down

dextran. Nordström & Hultin (1948) found that extracts from *Penicillium lilacinum* Thom, *P. funiculosum* Thom and *Verticillium coccorum* Westerdijk were able to break the 1,6-glucosidic linkages in dextran. The effect of faecal bacteria on dextran was studied in the present investigation (V) in order to try to isolate the split products. Fresh human faeces was mixed with dextran in different phosphate buffer solutions and the carbohydrate content estimated with the carbazol method. A rapid break down occurred at pH 6.24 while the break down at other pH was lower. There was no increase in reducing substances as estimated according to Somogyi (1937). As it seemed probable that the split products were organic acids, a dextran-faeces incubate was distilled after acidifying with sulphuric acid and the distillate electrometrically titrated with sodium hydroxide. When compared with an incubate of faeces with sodium chloride instead of dextran, it was found that the dextran-distillate was considerably more acid. Determination of the distillation constant according to Clark (1943) showed that some of the acid was acetic acid. Acetic acid was, however, present in both distillates. Other acids were also present in both cases but they have not yet been determined.

Summing up, it has been confirmed in the present investigation (V) that human faeces is able to break down dextran. Furthermore it has been proved (V) that the split products do not contain reducing substances but volatile acid substances.



### III. EFFECT OF DEXTRAN ON THE PLASMA PROTEINS.

When "Macrodex" is intravenously infused there will naturally occur a dilution of the blood. It was pointed out by *Thorsén* (1948) that the low erythrocyte values seen after dextran infusions in man were caused by dilution and not by anaemia. However, this author found, that the plasma proteins decreased more than could be counted for by dilution, and *Malmros & Thorsén* (*cit. Thorsén 1948*) found that the "depression" was largest for the albumin fraction of the plasma proteins. When large doses of the older Swedish dextran preparation, "Dextran Ph" were administered to animals, the serum protein value was lowered to 1—2 g./100 ml. *Bohmansson, Thorsén & Wilander* (1948) found, that the plasma proteins decreased proportionally to the amount of dextran administered. Clinically, the hypoproteinaemia following dextran infusions seemed of no importance (*Thorsén 1949, 1950*).

No effect on bleeding time or coagulability was seen by *Bohmansson, Thorsén & Wilander* (1948) when "Dextran Ph" was administered. *Laurell* (1951) further studied the influence of dextran on the conversion of fibrinogen to fibrin. She found, that (1) dextran does not affect the inactivation of thrombin at pH 7.3, (2) dextran is a fibrinoplastic substance which sensitizes the reaction between fibrinogen and thrombin, (3)

the fibrinoplastic effect varies with the molecular weight and the optimal effect is produced when the mean molecular weight is 75,000.

*Morrison & Bloom* (1951) administered large amounts of dextran (molecular weight about 75,000) to dogs. The animals died when the total amount of dextran was about 15 to 20 g. per kg. animal. At this point the haematocrit value was about 15 and the serum proteins diluted to the point at which clotting would not occur. The dogs were anaesthetized.

In the present investigation a study of the influence of dextran on the plasma proteins was performed (IV). 1000 ml. of "Macrodex" was administered intravenously to six healthy volunteers. The plasma proteins were determined according to *Theorell & Widström* (1931), and the values obtained were corrected for dilution, and the dilution was measured by haematocrit determinations according to *Hedin* (1890). There was in no single case a greater decrease of the plasma proteins than could be accounted for by dilution. Preliminary results by *Olhagen & Åberg* (1951, unpublished) have shown that the plasma protein fractions in man are equally diluted when 1000 ml. of "Macrodex" are intravenously administered.

The fractions were determined by electrophoresis, using the Tiselius' apparatus.

It may be concluded therefore, that (1) dextran dilutes the plasma, (2) the plasma proteins *in toto* as well as the fractions of

the plasma proteins are diluted when dextran is intravenously administered to man in doses of 1000 ml. The author has not studied the effect of larger dextran infusions on the plasma proteins in man.

#### IV. EFFECT OF DEXTRAN ON THE ERYTHROCYTE SEDIMENTATION RATE IN MAN.

##### 1. *In vitro* experiments.

That macro-molecules influence the erythrocyte sedimentation rate (E. S. R.) was established by *Nasse* (1836), *Fåhræus* (1921) and *Swedin* (1936). *Sulzer* (1950) studied the effect of various macro-molecular substances on the aggregation of red blood corpuscles and found that the effect was mainly due to the molecular weight of the substance and the viscosity of the solution. This author stated that the effect was due to adsorption.

The minimum mean molecular weight of dextran that can influence the E. S. R. was found by *Hint & Thorsén* (1950) to be 59,000. Their results were confirmed by *Hardwicke, Ricketts & Squire* (1950). *Thorsén & Hint* (1950) stated that dextran exerts its effect through a layer formed around each erythrocyte, and this layer was shown with photographs as fine threads between the erythrocytes.

Should an adsorption of dextran occur on the erythrocytes, it would be possible to show a decrease in a dextran solution if fresh human erythrocytes were added.

Such an experiment was performed in the present investigation (IV) but no decrease in the dextran content occurred. Furthermore, the erythrocytes, which had passed through the dextran solution were washed, but no

dextran could be washed from the surface of the cells. The same result was obtained when erythrocytes from a volunteer were taken and examined for dextran, after he had received dextran. The volunteer had an E. S. R. according to *Westergren* (1923) of 4 mm. per hour before the infusion, and 86 mm. per hour 6 hours after the infusion, at which point the blood sample examined was withdrawn (*Åberg 1951, unpublished*). That dextran does not enter the erythrocytes in man was found by *Bohmansson, Thorsén & Wilander* (1948) and later confirmed by *Ryttinger, Swedin & Åberg* (IV).

It has thus been found by other authors that dextran influences the suspension stability of the erythrocytes *in vitro* and that this effect is proportional *inter alia* to the mean molecular weight of the substance. This effect is, however, probably not an adsorption phenomenon (IV).

##### 2. *In vivo* experiments.

The earlier literature on the effect of Swedish clinical dextran preparations on the E. S. R. in man is somewhat confusing. *Grönwall & Ingelman* (1944) pointed out that dextran preparations for clinical use produce an increment in the E. S. R. The same result was obtained during the follo-

wing years by a number of investigators. However, differences in opinion regarding the correlation between the plasma concentration of dextran and the magnitude of the E. S. R. existed. For example, *Grönwall & Ingelman* (1945) stated that the E. S. R. of human blood *in vitro* varied with the plasma concentration of dextran, while *Wilander* (1946) did not find any such correlation *in vivo*.

As has been cited earlier, the manufacturers of dextran in Sweden have changed the molecular distribution of their preparation several times. This is probably the cause of the varying reports given as to the effect of "Dextran Ph" and "Macrodex" respectively on the E. S. R. For example, *Rosenqvist & Thorsén* (1951), stated that "Macrodex" has no effect on the agglutination of red blood cells.

The effect of "Macrodex" delivered to the Karolinska Sjukhuset in 1951 for use as a plasma volume expander was studied in the present investigation (IV) with regard to the E. S. R. in man. Six healthy volunteers were each given 1000 ml. of "Macrodex" intravenously and the E. S. R. was determined according to *Westergren* (1923) before the infusion as well as 1, 5, 12, and 24 hours after the infusion. The plasma concentration of dextran at the same time was determined according to *Hint & Torsén* (1947). It was found, that there was no correlation between the increment in the E. S. R. and the plasma concentration of dextran. There occurred in all cases an increment in the E. S. R.

This result might be explained as caused by the different paths of excretion used by the different molecules of dextran infused. The low molecular part will be rapidly excreted through the kidneys while the larger molecules seem to be eliminated, at least to some extent, with the gastric juice. The result will be that some hours after the infusion, the dextran in the blood consists of molecules larger than 36,000. *Thorsén & Hint* (1950) showed, that the low molecular part of dextran exerts a stabilizing effect on the suspension stability of the blood. Some hours after the infusion this stabilizing dextran has left the blood stream and the remaining part of the dextran consists of molecules with a greater influence on the E. S. R. Hence the maximum increment in the E. S. R. is seen several hours after the infusion according to *Ryttinger, Swedin & Åberg* (IV). The author wishes to stress that this explanation is only a theory. He has not performed any determinations on the molecular weight distribution of the dextran intravenously infused during the 4—5 days it remains in the blood. It is the author's opinion, that such an experiment should be performed, but this laboratory lacks the equipment for such an investigation.

Summing up, it has been shown in paper IV that dextran effects the E. S. R. in man. "Macrodex" delivered to the hospital in 1951 gave an increment in the E. S. R. which could not be correlated to the actual plasma concentration of dextran.



## SUMMARY.

The results obtained indicate, that some of the dextran intravenously administered to rabbits, was taken up by the spleen (I, II). Subcutaneously injected dextran exerted a positive chemotactic influence on the white blood cells in the rabbit (I). No uptake was seen in the liver or the muscle (II) or in the lungs (I) of this animal. In the kidneys dextran was histochemically found in the tubular epithelium (I), but it was not possible to draw any conclusions as to the significance of these findings, viz. if they signified excretion, reabsorption or storage (I). By intravenous infusion of dextrans with different mean molecular weights to rabbits and consequent analyses of kidney homogenates chemically, it was shown (I), that there occurred in the rabbit a transient accumulation of dextran in the kidneys. This was found also with dextrans with mean molecular weights, which according to polarimetric estimation of dextran in the urine, did not pass out into the urine.

Analyses of gastric juice samples from hospitalized patients who had received dextran ("Macrodex") intravenously, showed that there was a rise in the carbohydrate

content in the stomach after the infusion (III). Isolation and identification of this carbohydrate showed that it was probably dextran (III). It is pointed out, however, that the quantitative importance of this pathway of excretion remains to be shown.

It has furthermore been shown (V), that human faeces is able to break down dextran rapidly at pH 6.24 (phosphate buffer), and that the split products consist of volatile acid substances and that reducing substances are not the end product.

It has been shown (IV), that dextran in doses of 1000 ml. intravenously administered to man, dilutes the plasma. The plasma proteins *in toto* are diluted by this dose of dextran, and not depressed actively.

The effect of "Macrodex" *in vivo* on the erythrocyte sedimentation rate (E. S. R.) in man was studied in six healthy volunteers (IV) and it was found, that dextran influences the E. S. R., and that this influence is not parallel to the plasma dextran concentration and that the effect is probably not due to adsorption of dextran on the erythrocytes.

## REFERENCES.

- Bloom, W. L. & Willcox, M. L.: Determination of dextran in blood and urine. *Proc. Soc. Exper. Biol. & Med.* 76, 3, 1951.
- Bohmansson, G., Thorsén, G. & Wilander, O.: Dextran as a plasma substitute. *J. internat. chir.* 8, 890, 1948.
- Bull, J. P., Ricketts, C., Squire, J. R., Maycock, W. d' A., Spooner, S. J. L., Mollison, P. L. & Paterson, J. C. S.: Dextran as a plasma substitute. *Lancet*, Jan. 22, p. 134, 1949.
- Burson, N. & Bloom, W. L.: Studies on the gastrointestinal excretion of dextran. *Am. J. Med.* XI, 618, 1951.
- Cargill, W. H. & Brunner, H. D.: *Drug Trade News* 26, 46, 1951.
- Chambers, R. & Grand, C. G.: Chemotactic reaction of leucocytes to foreign substances in tissue cultures. *J. Cell. & Comp. Physiol.* 8, 1, 1936.
- Clark, E. P.: *Semimicro quantitative organic analysis*, p. 85, Academic Press, New York 1943.
- Durham, F. W., Bloom, W. L., Lewis, G. T. & Mandel, E. E.: *Public Health Report* 65, 670, 1950 *cit.* *Chemical Abstracts* 44, 7919 b, 1950.
- Engstrand, L. & Åberg, B.: Excretion of intravenously administered dextran. *Lancet*, June 10, p. 1071, 1950.
- Friberg, U., Graf, W. & Åberg, B.: On the histochemistry of the mast cells. *Acta path. et microbiol. Scandinav.* XXIX, 197, 1951.
- Friberg, U., Graf, W. & Åberg, B.: Toxicity study of chronic dextran administration to rabbits. In preparation, 1952.
- Fåhræus R.: The Suspension-stability of the blood. *Acta med. Scandinav.* 55, 1, 1921.
- Goldenberg, M., Crane, D. & Popper, H.: Effect of intravenous administration of Dextran, a macromolecular carbohydrate, in animals. *Am. J. Path.* 17, 939, 1947.
- Gray, I., Siiteri, P. K. & Pulaski, E. J.: Metabolism of plasma substitute I. Dextran (Macro-dex). *Proc. Soc. Exper. Biol. & Med.* 77, 626, 1951.
- Grotte, G., Knutson, R. C. & Bollman, J. L.: The diffusion of dextrans of different molecular sizes to lymph and urine. *J. Lab. & Clin. Med.* 38, 577, 1951.
- Grönwall, A.: Vortrag in der finnischen Gynäkologenvereinigung, Helsingfors 24.9.1949. Stenciled and distributed by Pharmacia Ltd., Uppsala, Sweden.
- Grönwall, A.: Dextran — ett nytt svenskt läkemedel II. Chockterapi med Macrodex — några allmänna synpunkter. *Svensk Kemisk Tidskrift* 63, 222, 1951.
- Grönwall, A. & Ingelman, B.: Några nya kolloidlösningar för infusionsändamål. *Nord. med.* 21, 247, 1944.
- Grönwall, A. & Ingelman, B.: Untersuchungen über Dextran und sein Verhalten bei parenteraler Zufuhr II. *Acta physiol. Scandinav.* 9, 1, 1945.
- Gurin, S. & Hood, D. B.: The identification and estimation of hexoses in polysaccharides and glycoproteins by the carbazol method. *J. Biol. Chem.* 131, 211, 1939.
- Hardwicke, J., Ricketts, C. R. & Squire, J. R.: Effect of dextran of various molecular sizes on erythrocyte sedimentation rate. *Nature* 166, 988, 1950.

- Hedin, S. G.: Der Hämatokrit, ein neuer Apparat zur Untersuchung des Blutes. *Skandinav. Arch. f. Physiol.* 2, 134, 1890.
- Hehre, E. J. & Sery, T. W.: Dextran-splitting anaerobic bacteria from the human intestine. *J. Bact.* 63, 424, 1952.
- Hildebrandt, F.: Untersuchungen über Dextran als Blutflüssigkeitsersatz. *Arztl. Wchnschr.* 5, 141, 1950.
- Hint, H. C. & Thorsén, G.: A micro method for determination of dextran in blood. *Acta chem. Scandinav.* 1, 808, 1947.
- Hotchkiss, R. D.: Microchemical reaction resulting in the staining of polysaccharide structure in fixed tissue preparations. *Arch. Biochem.* 16, 131, 1948.
- Ingelman, B.: Dextran and its use as a plasma-substitute. *Acta chem. Scandinav.* 1, 731, 1947.
- Ingelman, B.: Enzymatic break down of dextran. *Acta chem. Scandinav.* 2, 803, 1948.
- Ingelman, B.: Investigations on dextran and its application as a plasma substitute. *Upsala läkaref. Förh.* 54, 107, 1949.
- Ingelman, B.: Dextran — ett nytt svenskt läkemedel. I. *Svensk Kemisk Tidskrift* 63, 213, 1951.
- Ingelman, B. & Halling, M. S.: Some physico-chemical experiments on fractions of dextran. *Arkiv f. Kemi* 1, 61, 1949.
- Jorpes, E., Gardell, S., Werner, B. & Åberg, B.: On the heparin monosulfuric acid. Its staining with fuchsin-sulfurous acid after periodate oxidation. *Acta physiol. Scandinav.* 16, Suppl. 53, 36, 1948.
- Jorpes, E., Werner, B. & Åberg, B.: The fuchsin-sulfurous acid test after periodate oxidation of heparin and allied polysaccharides. *J. Biol. Chem.* 176, 277, 1948.
- Klevås, S.: Bestimmung des Dextrans im Blut bei seiner Anwendung als Plasmasubstitut. *Svensk Kemisk Tidskrift* 56, 262, 1944.
- Laurell, A.-B.: Influence of dextran on the conversion of fibrinogen to fibrin. *Scandinav. J. Clin. & Lab. Investigation* 3, 262, 1951.
- Mc Cutcheon, M.: Chemotaxis in Leucocytes. *Physiol. Rev.* 26, 319, 1946.
- Mc Manus, J. F. A.: Histological demonstration of mucin after periodic acid. *Nature* 158, 202, 1946.
- Morrison, J. L. & Bloom, W. L.: The effect of continuous infusion of hydrolyzed dextran on the cardiovascular system of the dog. *J. Pharmacol. & Exper. Therap.* 103, no. 4, December 1951.
- Morrison, J. L., Richardson, A. P. & Bloom, W. L.: The effects of antihistaminic agents on the reaction of the rat to dextran. *Arch. internat. de pharmacodyn. et de therap.* LXXXVIII, 98, 1951.
- Mowry, R. W., Longley, J. B. & Millican, R. C.: Histochemical demonstration of intravenously injected dextran in kidney and liver of the mouse. *J. Lab. & Clin. Med.* 39, 211, 1952.
- Nasse, H.: *Das Blut*. Bonn 1836.
- Nordström, L. & Hultin, E.: Dextranas, a new enzyme from mould. *Svensk Kemisk Tidskrift* 60, 283, 1948.
- Rosenqvist, H. & Thorsén, G.: Macrodex in the treatment of extensive burns. *Arch. Surg.* 62, 524, 1951.
- Schmitz, H.: Über das Schicksal des Macrodex im Organismus. *Klin. Wchnschr.* 23—24, 424, 1951.
- Schmitz, H.: Über Abbauvorgänge des Macrodex durch Leukozyten. *Med. Welt.* 20, 1350, 1951.
- Somogyi, M.: A reagent for the copperiodometric determination of very small amounts of sugar. *J. Biol. Chem.* 117, 771, 1937.
- Sulzer, R.: Agglutinating effect of macromolecular substances. *Helvetica physiol. pharmacol. acta* 8, 351, 1950. *cit. Chemical Abstracts* 45, 2036g, 1951.
- Swedin, B.: Untersuchungen über den Aggregationsmechanismus der Erythrozyten. *Biochem. Ztschr.* 288, 155, 1936.
- Theorell, H. & Widström, G.: Eine Methode zur Bestimmung von Fibrin, Globulin und Albumin im Blutplasma. *Zschr. f. d. ges. exper. Med.* 75, 692, 1931.
- Thorsén, G.: Dextran. Nyare undersökningar över preparatets egenskaper. *Nord. med.* 40, 2374, 1948.
- Thorsén, G.: Dextran as a plasma substitute. *Lancet*, Jan. 22, p. 132, 1949.
- Thorsén, G.: Resuscitative effect of Dextran Ph after large haemorrhages. *Acta chir. Scandinav.* 100, 221, 1950.

- Thorsén, G.: Lecture held at the N. R. C.'s Symposium on Burns in Washington, November 1950. National Academy of Sciences 1951.
- Thorsén, G. & Hint, H. C.: Aggregation, Sedimentation and Intravascular Sludging of Erythrocytes. *Acta chir. Scandinav. Suppl.* 154, 1950.
- Turner, F. P., Butler, B. C., Smith, M. E. & Scudder, J.: Dextran. An experimental plasma substitute. *Surg. Gynec. & Obst.* 88, 5, 1949.
- Weissberg, S. G. & Isbell, H. S.: National Bureau of Standards Report 1160, September 24, 1951.
- Westergren, A.: Zur Metodik der Senkungsreaktion. *Deutsche med. Wchnschr.* hft. 7, p. 218, 1923.
- Wilander, O.: Kliniska erfarenheter med dextran. *Nord. med.* 29, 344, 1946.
- Voorhees, A. B., Baker, H. J. & Pulaski, E. J.: Reaction of albino rats to injections of dextran. *Proc. Soc. Exper. Biol. & Med.* 76, 254, 1951.
- Zipf, R. E. & Waldo, A. L.: Spectrophotometric analysis of carbohydrates and study of anthrone reagent. *J. Lab. & Clin. Med.* 39, 497, 1952.







